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# Variational formulation, asymptotic analysis, and finite element simulation of wrinkling phenomena in modified plate equations modeling biofilms growing on agar substrates

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## Highlights

- Modeling biofilm over agar by modified Föppl–von Kármán plate equations (FvKEs).
- Finite element method based on minimax principle reduced to minimization.
- Boundary layer analysis of zero-bending solution of Monge–Ampère equation (MAE).
- Weak formulation of FvKEs separates geometrical and mechanical parts.
- Numerically obtained wrinkle patterns combine approximate cone–corona solutions.

## Abstract

The expansion of a bacterial biofilm on an agar substrate is modeled as a system of Föppl–von Kármán equations modified to include growth and coupling to a viscoelastic substrate. Analysis shows that wrinkles appear on the biofilm as a result of growth incompatibility and their frequency increases due to interaction with the agar layer. Simple cases of homogeneous radial and azimuthal growth are approximated by cone and corona solutions of the Monge–Ampère equation that are corrected by corner and boundary layers. A weak formulation of the problem allows us to express in-plane elastic strains and Airy potential solely in terms of the vertical displacement. We have developed a numerical method based on finite elements and simulated biofilm deformation for wide spectra of different growths. For heterogeneous growth, we find wrinkled patterns that are combinations of cones and coronas.

*Keywords:* Thin plates; Biological material; Wrinkles; Asymptotic analysis; Weak formulation; Finite element

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## 1. Introduction

Bacteria are one of the most widely spread life form in the world. They can be found everywhere on Earth: from volcanic geysers of Yellowstone National Park to subglacial antarctic lakes. However bacteria generally do not live as isolated entities but self-organize in colonies known as *biofilms*. These structures usually grow on moist air–surface or liquid–surfaces interfaces. They comprise bacterial cells embedded into a self-produced extracellular matrix (ECM) made out of exopolymeric substances (EPS), which occupy up to 90% of the colony volume, Flemming and Wingender [1]. Bacterial colonies are often undesirable for they may cause biocorrosion, Costerton et al. [2], and are responsible for spreading diseases and infections, Hobley et al. [3]. However other bacterial colonies can be useful for plant protection, Hobley et al. [3], or drug and fuel generation, Brenner et al. [4]. Understanding biofilm development can help to eliminate them when they are harmful and, on the contrary, enhance their growth when they are beneficial.

As many different processes take place in biofilms, their simulation is a complex problem. Since the ECM is crucial for biofilm growth, the colony shape and most bacterial activities, its study gives plenty of information about biofilm development. Bacteria use EPS for defending the colony in specified places and can increase its production by cell-to-cell communication, López et al. [5]. Wrinkles in the ECM form a network of channels that are used to bring substance from one side of colony to another when biofilm is too large to transport resources by diffusion, Wilking et al. [6]. The EPS also helps the colony to spread to larger areas, Seminara et al. [7].

There are different types of bacteria and, consequently, biofilms have different shapes. Bacterial communities growing in a stream organize themselves in mushroom-like systems, Rodríguez et al. [8], or form helical patterns in tubes, Espeso et al. [9]. Biofilms adhered to air–surface interface buckle and produce wrinkle patterns, Espeso et al. [10]. In our work we will focus on macroscopic development of biofilm growing on air–agar interface. An example is the bacterium *Bacillus subtilis*, which is widely used in experiments, Wilking et al., [11,6], Asally et al., [12], Seminara et al. [7]. This species is famous by its ability to organize complex wrinkling patterns during growth in a Petri dish. The wrinkles contain much information about the biofilm:

- The wrinkling network is a biofilm “fingerprint” because the geometry of the patterns depends on bacterial growth whereas their frequency and amplitude depend on properties of EPS and substrate, Espeso et al. [10].
- The colony uses wrinkles as a transport network. Thus understanding their emergence helps understanding how the colony infrastructure develops, Wilking et al. [6].
- Wrinkles are related to defensive mechanisms that bacteria use to survive. In particular, Asally et al. [12] have shown this for the cannibalism phenomenon that takes place in *Bacillus subtilis* colonies, López et al. [5].

Thus observed wrinkling patterns in bacterial biofilms depend on history and infrastructure of the colony and on properties of the EPS. Pattern formation is governed by microscopic cellular behavior, and macroscopic mechanical deformation.

In our work we follow the ideas of Espeso et al. [10] and consider the biofilm as a thin solid plate bonded to a viscoelastic substrate. The biofilm corrugates because of growth. We include heterogeneity of material properties (thickness and Young modulus) in the derivation of modified Föppl–von Kármán equations (FvKEs) carried out by Dervaux et al. [13]. To derive equations of agar substrate motion, we use a scaling method that is simpler than that by Huang [14] but leads to the same results. We couple both systems of equations to write the full mathematical model of ECM deformation. Performing asymptotic analysis of the full system in the simple case of pure radial growth (that has a cone solution), we show that, apart of a corner layer near the cone tip described by Audoly and Pomeau [15], there appears a boundary layer at the rim due to biofilm incompressibility. Because of the insufficient smoothness of solutions, we consider the weak formulation of the problem respect of vertical displacement and the Airy potential as done by Jones and Mahadevan [16]. However, we can further simplify the resulting problem by expressing the Airy potential as a function of the vertical displacement, which thereby becomes the only unknown. This approach allows us to develop an efficient numerical method based on finite elements, Hughes [17], Zienkiewicz and Taylor [18], and simulate biofilm development for wide spectra of homogeneous and heterogeneous growths. Another advantage of our weak formulation is that we explicitly separate a geometrical part corresponding to the Monge–Ampère equation (MAE) and a mechanical part representing bending energy. The weak form of MAE has infinitely many solutions but the bending energy term regularizes it providing a well defined solution.

The present paper has the following structure. In Section 2 we consider the wrinkling phenomena in different applications and extract the main features responsible for the formation of patterns. Next, in Section 3, we present the